

Chelating Rotaxane Ligands as Fluorescent Metal Ion Sensors

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Abstract: Although metal ion-binding interlocked molecules have been under intense investigation for over three decades, their application as scaffolds for the development of sensors for metal ions remains under-explored. Here we demonstrate the potential of simple rotaxane scaffolds as metal ion-responsive ligand scaffolds through the development of a proof-of-concept selective sensor for Zn^{2+} .

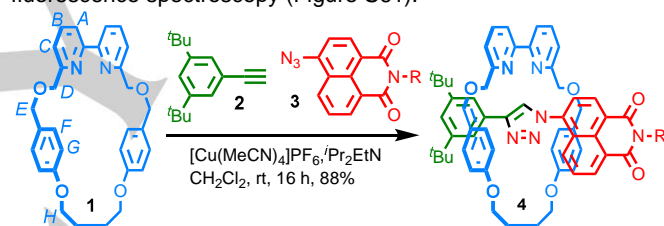
Small molecule fluorescent probes are powerful tools for visualizing metal ions in living systems due to their rapid response time and potential for non-invasive, high resolution and quantitative imaging.^[1] In particular, the development of small molecule sensors^[2,3] for the detection and quantification of Zn^{2+} *in vivo* has attracted considerable recent attention due to the spectroscopically silent nature of the d^{10} Zn^{2+} ion, combined with the recognition that changes in zinc homeostasis are associated with diseases with high morbidity such as Alzheimer's disease,^[4] Type II diabetes,^[5] and age related macular degeneration.^[6]

Such small molecule probes are generally composed of a multi-dentate chelating ligand linked to a fluorophore whose output is modulated by the metal binding event. Mechanically interlocked molecules,^[7] particularly those synthesized using metal-mediated approaches,^[8] often possess a well-defined binding pocket containing multiple donor atoms for metal ions.^[9] Such multi-dentate "mechanically chelating" ligands^[10] seem ideal for the development of metal-selective ligands and related metal ion sensors by exploiting the size and shape of the three dimensional cavity formed by the mechanical bond. However, almost all interlocked molecules that display a fluorescent response^[11] upon metal binding rely on large amplitude motion in relatively structurally complex molecular shuttles.^[12,13,14] Furthermore, in most cases, the selectivity between competing analytes is not reported. Indeed, to our knowledge only one example has been reported in which the mechanical bond is used to generate a metal binding pocket to report the binding of competing analytes; in 2004 Hiratani and co-workers disclosed a [1]rotaxane that selectively binds Li^+ over Na^+ and K^+ and reports metal binding through a "switch on" fluorescence response.^[15,16]

Given that the synthesis of mechanically chelating ligands is now relatively simple, it is perhaps surprising that these scaffolds have been overlooked in the development of cation sensors, particularly as a related strategy for the sensing of anions has been developed by Beer and co-workers.^[17] Thus, we set out to demonstrate the potential of the mechanical bond as a structural motif in the development of selective metal ion

sensors through the development of a proof-of-concept selective sensor for Zn^{2+} . Herein we report that not only is this approach successful, but that relatively small structural changes in the axle component lead to large changes in the photophysical response to divalent metal ions.

We synthesized rotaxane **4**^[18] in excellent isolated yield (88%) using the active template^[19] Cu-mediated alkyne-azide cycloaddition (AT-CuAAC) reaction^[20,21] between azido fluorophore **3** and acetylene **2** in the presence of readily available bipyridine macrocycle **1**^[22] and $[\text{Cu}(\text{MeCN})_4]\text{PF}_6$. Addition of one equivalent of $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ to a solution of **4** in CD_3CN resulted in large changes in the ^1H NMR spectrum (see ESI Figure S39) consistent with the binding of the metal ion into the cavity of the macrocycle, confirming that **4** is capable of acting as a ligand. The behavior of rotaxane **4** as a metal-responsive sensor for Zn^{2+} was investigated by fluorescence titration with $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$. Portion-wise addition of Zn^{2+} to rotaxane **4** in MeCN led to a monotonic quenching of the emission at 560 nm^[23] that plateaued once a full equivalent had been added (Figure 1a). In contrast, titration of the non-interlocked axle with Zn^{2+} revealed no change by UV-vis or fluorescence spectroscopy (Figure S61).^[24]



Scheme 1. Synthesis of fluorescent rotaxane **4** using the AT-CuAAC reaction. $\text{R} = \text{CH}_2\text{C}(\text{H})\text{Ph}_2$.

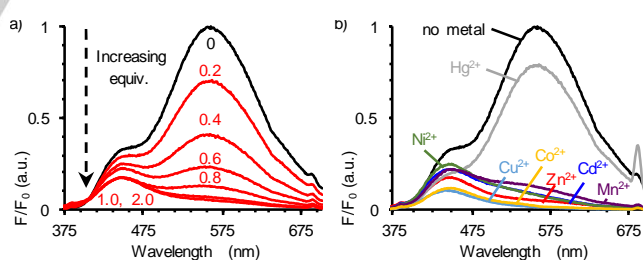


Figure 1. Emission profile of rotaxane **4** (MeCN, 100 μM , $\lambda_{\text{ex}} = 343$ nm) a) in the presence of varying equivalents of $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, b) in the presence of $\text{M}(\text{ClO}_4)_2$ (5 equiv.). No emission was observed in the case of $\text{Fe}(\text{ClO}_4)_2$.

Rotaxane **4** is a candidate fluorescent "switch off" sensor for Zn^{2+} . However, examination of the selectivity of this response revealed a complete lack of discrimination between Zn^{2+} and selected divalent metal cations; addition of $\text{M}(\text{ClO}_4)_2$ ($\text{M} = \text{Mn}^{2+}$, Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Cd^{2+} , Hg^{2+}) to a solution of **4** led to quenching of the emission to a greater or lesser extent than that observed with Zn^{2+} (Figure 1b).^[23] Thus, **4** cannot be classed as a metal ion sensor as, although it responds to metal ion binding, it cannot discriminate between competing analytes.

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Having confirmed that binding of metal ions within the cavity of the rotaxane can, in principle, lead to an optical response, we extended our investigation to heteroatom-substituted naphthalimide rotaxanes **5-7** (Figure 2), readily synthesized in good to excellent yield (62%, 72% and 86% respectively, see ESI). These were selected as the heteroatoms can potentially interact directly with the metal ion and are known to significantly alter the photophysical properties of naphthalimide fluorophore.^[25] ¹H NMR spectroscopy (Figures S39-41) confirmed that rotaxanes **5-7** act as ligands for Zn²⁺ and UV-vis titration revealed excellent goodness of fit to a 1 : 1 binding isotherm; addition of Zn(ClO₄)₂·6H₂O resulted in the appearance of absorbance bands at 312 and 322 nm corresponding to the metal-bound bipyridine moiety (Figures S46-48).^[26]

The fluorescence response of rotaxanes **5-7** to binding of selected divalent metal ions revealed very different behavior. Similar to rotaxane **4**, rotaxane **5** displays a simple switch off response to Zn²⁺ and the majority of other metal ions investigated (Figure 2a) although the extent of quenching varied considerably with metal ion. In contrast, rotaxane **6** (Figure 2b) displays little or no response to Mg²⁺, Ca²⁺ and Mn²⁺, a switch off response of varying degree to Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺ and Cd²⁺ and a weak switch on response to Fe²⁺. Strikingly, Hg²⁺ produced a significant switch on response, suggesting that rotaxane **6** is a good starting point for the development of an Hg²⁺ selective sensor. Pleasingly, addition of Zn²⁺ to rotaxane **7** triggers a switch on response with a concomitant blue shift in the emission of 15 nm. All other metal ions, with the exception of Cd²⁺, which produces a switch on response and blue shift of 8 nm, produce a switch off or no response (Figure 2c). Competition experiments demonstrate that in many cases Zn²⁺ is also able to displace metal ions from ligand **7**; addition of M²⁺ followed by Zn²⁺ to **7** resulted in recovery of fluorescence in the case of Mg²⁺, Ca²⁺, Mn²⁺, Fe²⁺ and Co²⁺.

Having identified rotaxane **7** as a switch on sensor for Zn²⁺, albeit with Cd²⁺ as a confounding analyte,^[27] we investigated its behavior in more detail. The ¹H NMR spectrum of **7** (Figure 3b) displays a number of differences with the corresponding non-interlocked axle (Figure 3a), in particular triazole proton H_m appears significantly deshielded in the interlocked structure by Δδ = 1.36 ppm, consistent with the expected C-H...N hydrogen bond with the bipyridine moiety,^[28] and alkyl protons H_k and H_l appear at lower ppm, suggesting that they engage in C-H...π contacts with the aromatic rings of the macrocycle. Crystals of **7** suitable for X-ray analysis were grown by slow evaporation from MeCN. The solid-state structure largely is consistent with the interactions proposed to be present in solution (Figure 4a); triazole proton H_m is engaged in a C-H...N hydrogen bond with the bipyridine nitrogens, as is one of H_k, and one each of protons H_k and H_l are in close contact with the phenyl rings of the macrocycle. Crystals of **7** suitable for X-ray analysis were grown by slow evaporation from MeCN. The solid-state structure largely is consistent with the interactions proposed to be present in solution (Figure 4a); triazole proton H_m is engaged in a C-H...N hydrogen bond with the bipyridine nitrogens, as is one of H_k, and one each of protons H_k and H_l are in close contact with the phenyl rings of the macrocycle.

Portion-wise addition of Zn(ClO₄)₂·6H₂O to **7** in CD₃CN led to broadening of the ¹H NMR resonances corresponding to **7** and the appearance of a new set of resonances assigned to [Zn(**7**)]²⁺ (Figure S42). Once one equivalent of Zn²⁺ had been added (Figure 3c) no further change was observed. Strikingly, in addition to the expected deshielding of bipyridine resonances H_A, H_B, and H_C, the triazole and alkyl resonances shift significantly on Zn²⁺ binding; H_m is observed at 5.52 ppm in the metal complex (Δδ = −3.84 ppm) suggesting it is considerably shielded relative to the non-interlocked axle, while H_k and H_l shift to higher ppm suggesting that the shielding C-H...π interactions are interrupted.

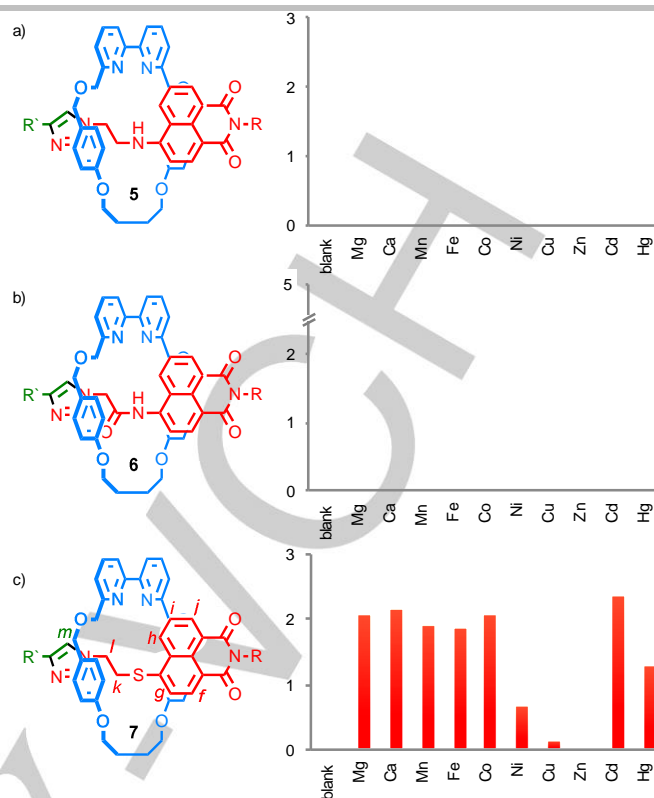


Figure 2. Rotaxanes a) **5** ($\lambda_{\text{ex}} = 435$ nm), b) **6** ($\lambda_{\text{ex}} = 380$ nm) and c) **7** ($\lambda_{\text{ex}} = 379$ nm) and their fluorescence response to 5 equiv. M(ClO₄)₂ (MeCN, 100 μM) (blue bars). Red bars in c refer to the fluorescence response on sequential addition of M²⁺ followed by Zn²⁺ (5 equiv. each). R = CH₂C(H)Ph₂, R' = 3,5-di-*t*-Bu-C₆H₃.

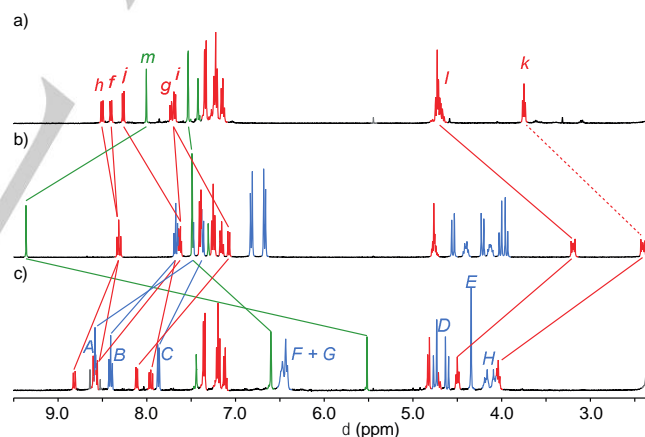


Figure 3. Partial ¹H NMR spectra (CD₃CN, 400 MHz, 298 K) of a) the non-interlocked axle of rotaxane **7**; b) rotaxane **7** and c) rotaxane **7** + Zn(ClO₄)₂·6H₂O. For labelling scheme see Scheme 1 (macrocycle) and Figure 2 (axle).

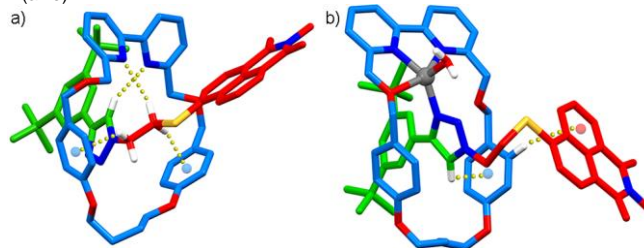


Figure 4. Solid state structures of a) rotaxane **7** (selected distances in Å: C-H_m...N = 2.46, C-H_k...N = 2.71, C-H_k...π = 2.66, C-H_l...π = 2.79; dihedral angle C_k-S-C-C_{ipso} = 8.2°) and; b) [Zn(**7**)]²⁺ (selected distances: C-O...Zn = 2.28, C-H_m...π = 2.74, C-H_l...π = 2.86; dihedral angle C_k-S-C-C_{ipso} = 29.9°).

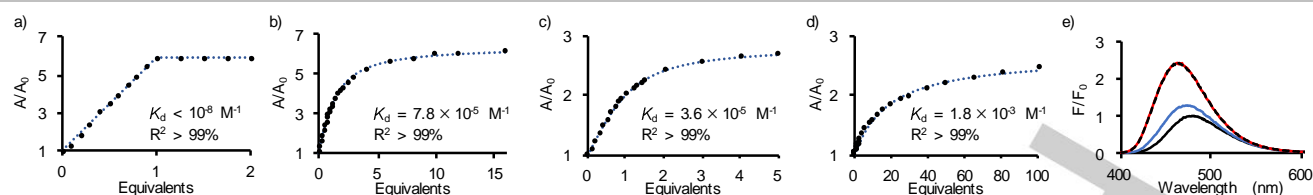


Figure 5. UV-vis ($\lambda = 322$ nm) titrations of **7** (100 μM) with $M(ClO_4)_2$ as a function of a) Zn^{2+} , b) Zn^{2+} (2% H_2O -MeCN), c) Cd^{2+} (MeCN), d) Cd^{2+} (2% H_2O -MeCN). e) Emission spectrum ($\lambda_{ex} = 380$ nm) of **7** in 2% H_2O -MeCN (black), + 5 equiv. Cd^{2+} (blue), + 5 equiv. Zn^{2+} (red), + 5 equiv. each Zn^{2+} and Cd^{2+} (black dashed).

The solid-state structure of $[Zn(\mathbf{7})](OTf)_2$ obtained by slow evaporation of a MeCN solution confirms the coordination of Zn^{2+} within the macrocycle cavity and exhibits a number of features consistent with the solution state 1H NMR data. The Zn^{2+} ion is coordinated by the bipyridine and triazole N-donors alongside one of the aliphatic ether O-donors and a water molecule.^[29] This coordination interrupts the triazole C-H \cdots N hydrogen bond and this proton is now engaged in a shielding C-H \cdots π interaction with one of the macrocycle phenyl rings. The shielding C-H \cdots π interactions of H_k and H_l are also interrupted. Interestingly, in the solid state, coordination leads to the appearance of a C-H \cdots π interaction between one of the aromatic phenyl ring protons and the naphthalene rings and a large change in the dihedral angle about the S-naphthyl bond. With the obvious caveat that the solid state structure of $[Zn(\mathbf{7})]^{2+}$ is not necessarily representative of the solution state (co)conformation, the observed changes in the interactions between the macrocycle and fluorophore component, along with the changes in the conjugation between the S-donor and the naphthalene core concomitant with the altered dihedral angle suggests that the changes in emission properties of **7** on metal binding may be due to changes in the (co)conformation of the ensemble.

The binding of rotaxane **7** with Zn^{2+} was determined to be extremely strong ($K_d < 10^{-8} M^{-1}$) by UV-vis titration^[30] with non-linear regression analysis (Figure 5a), stronger than that of macrocycle **1** alone ($K_d = 8.9 \times 10^{-8} M^{-1}$; Figure S57) suggesting that the mechanically chelating triazole ligand significantly enhances Zn^{2+} binding in **7**.^[31] Conversely, the binding of rotaxane **7** with Cd^{2+} ($K_d = 3.6 \times 10^{-5} M^{-1}$; Figure 5b), is about three orders of magnitude weaker than that of macrocycle **1** alone ($7.5 \times 10^{-8} M^{-1}$; Figure S59). The difference in binding strength for **7** with Zn^{2+} and Cd^{2+} was further corroborated by 1H NMR titration. In the presence of 1 equiv. of both Zn^{2+} and Cd^{2+} , $[Zn(\mathbf{7})]^{2+}$ is observed to form selectively (Figure S43), whereas the same experiment with macrocycle **1** produced a 4 : 1 mixture of Zn^{2+} and Cd^{2+} complexes (Figure S45). These results demonstrate that the mechanical bond imparts a significant degree of selectivity on the binding of otherwise similar metal ions, perhaps due to the different sizes of the Zn^{2+} and Cd^{2+} ions (88 pm vs 109 pm respectively)^[32] or by sterically excluding additional ligands from the coordination sphere of the metal ion.

The stronger binding of Zn^{2+} compared with Cd^{2+} allowed us to use a more competitive solvent mixture (2% water in MeCN) to impart selectivity on sensor **7**. In the presence of H_2O (Figures 5c-d), the binding of both Zn^{2+} and Cd^{2+} **7** was diminished ($K_d = 7.8 \times 10^{-5}$ and $1.8 \times 10^{-3} M^{-1}$ respectively). As a result, whereas 1 equiv. of Zn^{2+} achieved 50% of F_{max} and saturation was achieved at ~ 12 equiv., Cd^{2+} required ~ 20 equiv. to achieve 50% switch on and ~ 100 equiv. to achieve saturation, demonstrating that under these conditions Cd^{2+} is not bound by **7** to a significant extent. Thus, whereas addition of 5 equivalents

of Cd^{2+} to **7** in MeCN- H_2O leads to a weak response, when Zn^{2+} is added to the same solution, the expected switch on of luminescence is observed, demonstrating selectivity for Zn^{2+} over Cd^{2+} (Figure 5e).

In conclusion, we have demonstrated that relatively simple interlocked molecules can provide an excellent scaffold for the design of metal ion sensors. Importantly we show that the binding pocket provided by the mechanical bond can impart not only an optical response but also a degree of binding selectivity, as in the case of rotaxane **7**. It is also noteworthy that, in addition to sensor **7**, which shows the desired Zn^{2+} selective response in MeCN- H_2O , rotaxane **6** also appears to show a selective switch on response, in this case to Hg^{2+} .^[33] The mode of switching, at least in the case of **7**, appears to be the reorientation of the components on metal binding altering the relative positions of the fluorophore and the macrocycle leading to an enhancement of fluorescence, but this requires more detailed investigation. The origin of the different behaviors of **4-6** also requires further investigation; as it appears that the different linker units are not directly involved in the binding event, it seems likely that the specific photophysical properties of the fluorophore unit are important.^[25] Practically, the next step in the development of interlocked hosts for the detection of metal ions in biological systems is to render them water soluble, a task which is ongoing in our laboratories, greatly facilitated by the synthetic flexibility of the AT-CuAAC reaction. More generally, the results presented here suggest that, although interlocked molecular machines remain an exciting and important direction for the field, the use of the mechanical bond as a structural feature, for instance in the design of mechanically chelating ligands, has the potential to lead to new developments in a range of areas.

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Keywords: rotaxane • sensor • fluorescent • mechanical bond • ligand

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of fit ($R^2 = 98.2\%$; Figures S49-50). The discrepancy may be due to the water associated with the metal salt; the same procedure in the presence of 2% H_2O gave comparable values for K_d (Figures S51-2).

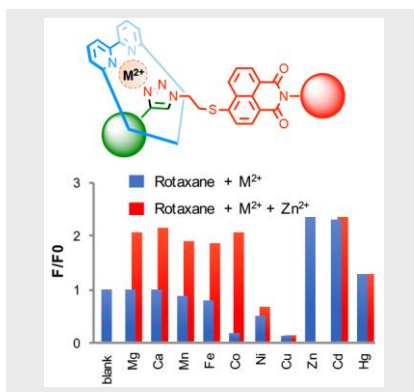
- [31] **4**, **5** and **6** exhibit similar or weaker binding with Zn^{2+} than **1**. In the case of **4** this be caused by the sterically crowded binding motif. The weaker binding of **5** and **6** probably reflects the $N-H\cdots N$ H-bond interactions (implied by 1H NMR) to be overcome in binding the metal ion.
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A ring to bind them. Although many mechanically chelating rotaxanes and catenanes have been reported in which donor atoms bridge the mechanical bond to bind metal ions, their use as scaffolds for the selective detection of cations remains under-explored. Here we demonstrate that this simple strategy is effective for the development of a fluorescent metal responsive probe.



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**Chelating Rotaxane Ligands as
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